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Developing Chicken Embryo-Report of the in-house investigations of ~~Ammonium~~
Alginate in the developing chicken embryo 1/31/75 ~~_____~~

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MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TO : Mr. Alan Spiher
GRAS Review Branch, HFF-335

DATE: January 31, 1975

THRU : Dr. Herbert Blumenthal, Acting Director
Division of Toxicology, HFF-150

FROM : M. Jacqueline Verrett, Ph.D.
Reproductive Physiology Branch, HFF-157



SUBJECT: Investigation of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo.

Attached is the report of the in-house investigations of Ammonium Alginate in the developing chicken embryo.

Investigations of the Toxic and Teratogenic Effects of
GRAS Substances to the Developing Chicken
Embryo: Ammonium Alginate

Protocol:

Ammonium Alginate (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in corn oil as the solvent by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2,3).

Groups of fifteen or more eggs were treated under these four conditions at several dose levels until a total of seventy-five to one hundred eggs per level was reached for all levels allowing some hatch. Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in tables 1 through 4 for each of the four conditions of test.

Column 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i.e., total non-viable divided by total treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia or other nerve disorders.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Column 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and the untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data of columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

Discussion:

Treatment via the air displayed no toxicity of ammonium alginate above the corn oil background. Yolk treatment at 0 hours led to a significant regression of mortality versus dose with a calculated LD₅₀ of 1.815 mg/kg (0.0908 mg/egg) indicating a rather high toxicity for this test condition. There was no linear relationship obtained for yolk treatment at 96 hours.

Scattered abnormalities were observed for all four conditions of test, but in no instances were these different from or significantly higher than that observed in the corn oil treated or untreated controls. Ammonium alginate displayed no teratogenicity in the test conditions employed.

1. Ammonium Alginate, Calco Co., San Diego, California.
2. McLaughlin, J., Jr., Marliac, J.-P., Verrett, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O.G., (1963) Toxicol. Appl. Pharmacol. 5, 760-770.
3. Verrett, M.J., Marliac, J.-P., and McLaughlin, J., Jr., (1964) JAOC 47, 1002 - 1006.
4. Finney, D.J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendix I.

Table 1

Ammonium Alginate

Air Cell @ 0 Hours

Dose		Number of Eggs	** Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
0.5000	10.000	120	66.66	3.33	0.83
0.2000	4.000	94	56.38	3.19	1.06
0.1000	2.000	95	50.52	5.26	2.10
0.0500	1.000	120	71.66*	3.33	0.83
0.0200	0.400	120	64.16	2.50	3.33
Corn Oil		145	58.62	1.37	2.06
Control		375	27.46	0.53	0.53

* Significantly different from solvent $p \leq 0.05$

** Slope is negative

Table 2

Ammonium Alginate

Air Cell @ 96 Hours

Dose		Number of Eggs	** Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
0.6250	12.500	85	22.35	3.52	2.35
0.2500	5.000	85	35.29	2.35	1.17
0.1250	2.500	83	43.37*	3.61	1.20
0.06250	1.250	85	32.94	1.17	1.17
0.0250	0.500	85	35.29	1.17	1.17
Corn Oil		124	25.80	1.61	0.80
Control		375	27.46	0.53	0.53

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Table 3

Ammonium Alginate

Yolk @ 0 Hours

Dose		Number of Eggs	** Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
1.250	25.000	120	81.66*	0.83	0.00
0.500	10.000	120	87.50*	0.83	0.83
0.250	5.000	120	78.33*	1.66	0.83
0.1250	2.500	120	68.33*	1.66	0.00
0.0500	1.000	120	59.16*	1.66	0.00
Corn Oil		150	32.66	0.00	0.00
Control		375	27.46	0.53	0.53

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** LD₅₀ 1.8151 mg/kg (0.09075 mg/egg)

Ammonium Alginate

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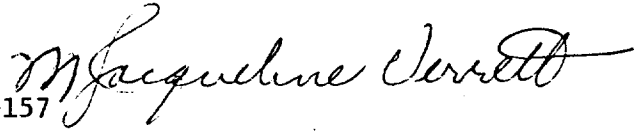
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